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Systematic review of the firefly genus *Emeia* Fu, Ballantyne & Lambkin, 2012 (Coleoptera, Lampyridae) from China

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Abstract

The Luciolinae genus *Emeia* Fu, Ballantyne & Lambkin, 2012 is reviewed. Phylogenetic relationships based on *cox1* DNA barcoding sequences from 42 fireflies and 2 outgroup species are reconstructed. The dataset included three main Lampyridae subfamilies: Luciolinae, Photurinae and Lampyrinae, and *Emeia* was recovered within Luciolinae. A new species, *Emeia pulchra* Zhu & Zhen **sp. nov.**, is described from the wetland of Lishui, Zhejiang, China. *Emeia pulchra* is sister species to *E. pseudosauteri* from Sichuan, which is supported by morphological characters and a phylogeny based on DNA barcoding sequences. The two species are separated geographically as shown on the distribution map. A key to species of *Emeia* using males is provided.

Keywords

Cytochrome c oxidase subunit I , DNA barcoding, *Emeia*, firefly, Lampyridae

Introduction

Emeia Fu, Ballantyne & Lambkin, 2012 (Luciolinae) was established as a monotypic genus (Fu et al. 2012) with *Emeia pseudosauteri* (Geisthardt 2004) as the type species. *Emeia pseudosauteri* was first described from Mount Emei, Sichuan, China by Michael

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Geisthardt in the genus *Curtos* Motschulsky, 1845 (Geisthardt 2004), and then transferred to *Emeia* based on morphological evidence (Fu et al. 2012). The genus *Emeia* Fu, Ballantyne & Lambkin had only one species (*E. pseudosauteri*) recorded in China before this study. The primary phenotypic feature of *Emeia* was the trilobite-like larva. The thoracic and abdominal terga of *Emeia* larvae are distinct. The lateral thoracic tergal margins are broad, similar to those of a trilobite "cephalon", while the abdomen is narrow and curls ventrad in the posterior part. At present, definition of the genus *Emeia* is based on the morphology of *E. pseudosauteri*, which makes it insufficient in light of the discovery of a second species.

In this study, based on specimens collected from Lishui, Zhejiang, China, we describe adults of *Emeia pulchra* Zhu & Zhen sp. nov. based on morphological and molecular data. We compare it with the previously described *E. pseudosauteri*. We also provide new information on the adult male hind wing venation of the type species *E.pseudosauteri.* With our detailed examination of both species, we present a systematic review of the genus *Emeia* and a key to species.

Materials and methods

Abbreviations

EL elytral length;
EW elytral width;
PL pronotal length;
BL body length (the sum of PL, EL and the length of the exposed portions of the head from the pronotum);
BW body width (the greatest distance across the elytra, BW=2EW);
T7, 8 abdominal tergite numbers;
V6, 7 abdominal ventrite numbers.

Adult males of *Emeia pulchra* Zhu & Zhen sp. nov. were collected from Jiulong National Wetland Park, Lishui, Zhejiang Province in April, 2020. The holotype and paratypes of the new species are stored at School of Life Sciences, Westlake University, Hangzhou, Zhejiang. Samples of both male and female *Emeia pseudosauteri* were collected from Mt. Tian Tai, Sichuan Province in April, 2021.

Habitus images were taken using a Nikon D7500 camera. Images of genitalia were taken using a Nikon D7500 camera mounted on an SZ650 microscope (Chongqing Optec Instrument Co., Ltd.) under reflection or transmission light. Images were edited using Adobe Photoshop CS6. Morphological terminology and measurements follow those decribed in Douglas (2017). The body length (**BL**) is the sum of the pronotal length (**PL**) and elytral length (**EL**) plus the length of the exposed portions of the head from the pronotum. The abbreviations **EW** and **BW** (**BW=2EW**) denote elytral width and body width, respectively (Fig. 1A). The length

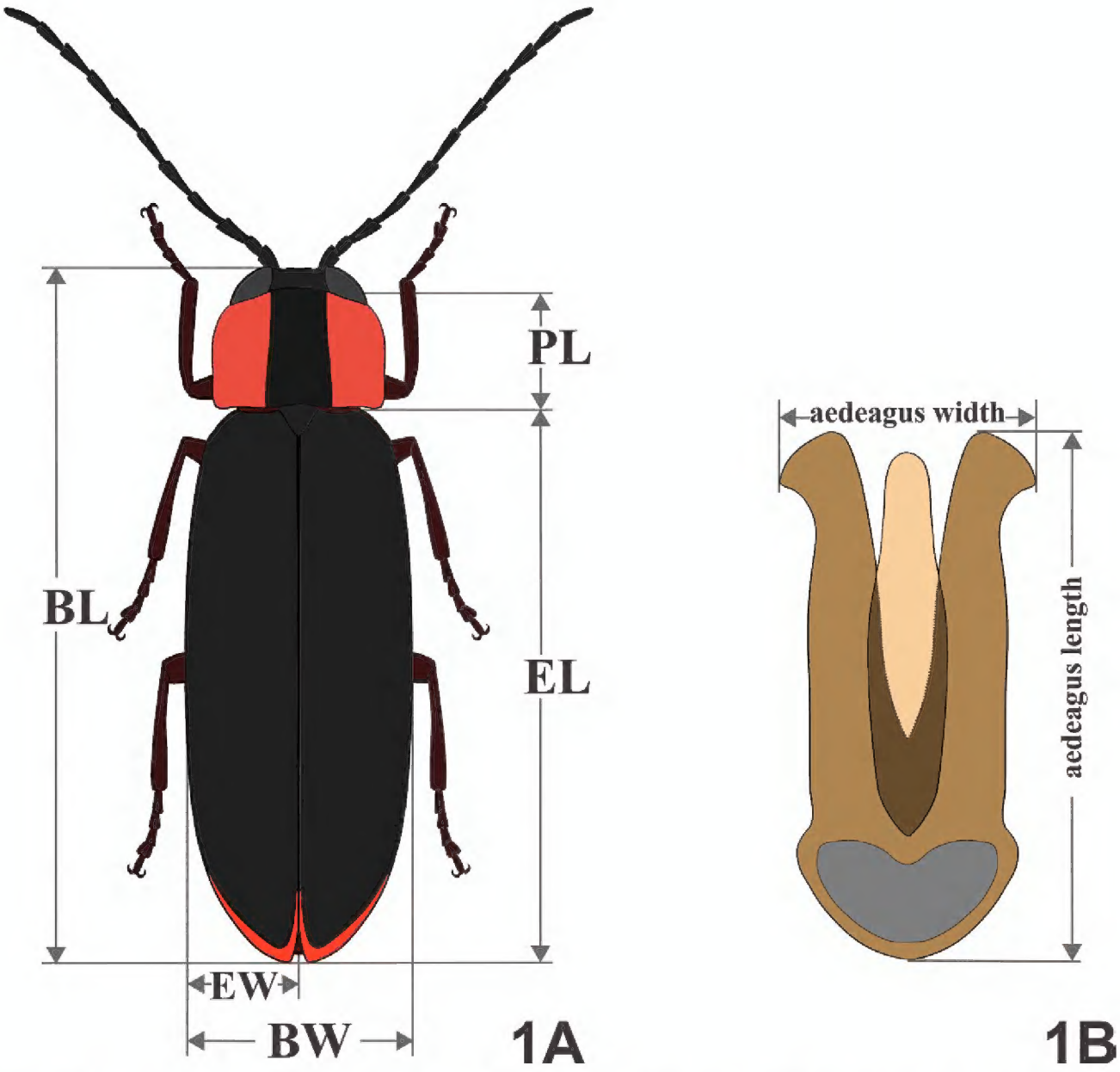


Figure 1. Measurement methods and terminology **A** male habitus, dorsal view **B** aedeagus, ventral view.

and width of the aedeagus and aedeagal sheath were measured under the microscope using the OLYMPUS cellSens Dimension software (v 3.1.1) (Fig. 1B). The dissected aedeagus and aedeagal sheath structures are preserved in pure glycerol in small vials with the corresponding specimens.

We sequenced the *cox1* gene barcode fragment from *Emeia pseudosauteri* and *E. pulchra*. Specifically, total DNA of the two *Emeia* species was isolated using the DNeasy Blood and Tissue Kit (Zhejiang Easy-Do Biotech CO., Ltd.), according to the manufacturer's protocol. The primers LCO 1490 and HCO 2198 (Folmer et al. 1994) were used to amplify the barcode fragments of the mitochondrial gene cytochrome c oxidase subunit I (*cox1*). We performed the PCR reaction in a 25 μL reaction mix containing 1× PCR buffer, 1 μL of each primer in a final concentration of 1 μM, 1 μL of template, 0.2 mM of each dNTP and 0.5 units of Taq polymerase (Takara Biomedical Technology CO., Ltd). The PCR thermal regime consisted of an initial denaturation at 95 °C for 3 min; 30 cycles of 30 s at 94 °C, 30 s at 48 °C and 30 s at 72 °C, followed by a 5 min final extension at 72 °C. PCR products were checked by electrophoresis in 1% agarose gel at 170 V for 20 min, and visualized under a UV transilluminator with nucleic acid dye (Cofitt Life Science, Hong Kong). The PCR products were cleaned

using Easy Gel Extraction & Clean-up kit (Zhejiang Easy-Do Biotech CO., Ltd.). The cleaned products were sequenced with an ABI 3730XL sequencer (Applied Biosystems, California, USA) by Zhejiang Sunya Biotechnology Co., Ltd.

MEGA6 (Tamura et al. 2013) was used for phylogenetic reconstruction. *Cox1* barcode sequences from three main subfamilies, *i.e.*, Luciolinae, Photurinae and Lampyrinae, were included, and sequences from the family Rhagophthalmidae were used as an outgroup (Table 1). The maximum likelihood method was used with 1000 boot-

Table 1. Genbank accession numbers for *cox1* sequences used for the phylogenetic analysis.

Species	Family	Sub-family	GenBank id
Pyrocoelia pectoralis	Lampyridae	Lampyrinae	KP763467.1
Pyrocoelia rufa	Lampyridae	Lampyrinae	AF452048.1
Pyrocoelia abdominalis	Lampyridae	Lampyrinae	AB608766.1
Pyrocoelia atripennis	Lampyridae	Lampyrinae	AB608767.1
Pyrocoelia discicollis	Lampyridae	Lampyrinae	AB608768.1
Pyrocoelia fumosa	Lampyridae	Lampyrinae	AB608769.1
Pyrocoelia matsumurai	Lampyridae	Lampyrinae	AB608770.1
Diaphanes nubilus	Lampyridae	Lampyrinae	MG200080.1
Diaphanes pectinealis	Lampyridae	Lampyrinae	NC_044793.1
Photinus pyralis	Lampyridae	Lampyrinae	KY778696.1
Ellychnia corrusca	Lampyridae	Lampyrinae	KR483038.1
Ellychnia hatchi	Lampyridae	Lampyrinae	JF887410.1
Pyractomena lucifera	Lampyridae	Lampyrinae	MF640134.1
Pyractomena borealis	Lampyridae	Lampyrinae	HQ928227.1
Pyractomena angulata	Lampyridae	Lampyrinae	JN290381.1
Aspisoma sp.	Lampyridae	Lampyrinae	EU009322.1
Lucidina accensa	Lampyridae	Lampyrinae	AB608771.1
Lucidina kotbandia	Lampyridae	Lampyrinae	FJ462784.1
Lucidota atra	Lampyridae	Lampyrinae	HQ984304.1
Photuris pensylvanica	Lampyridae	Photurinae	MF634963.1
Photuris quadrifulgens	Lampyridae	Photurinae	HM433520.1
Bicellonycha lividipennis	Lampyridae	Photurinae	KJ922151.1
Bicellonycha wickershamorum	Lampyridae	Photurinae	EU009302.1
Pristolycus sp.	Lampyridae	Luciolinae	MK292099.1
Sclerotia flavida	Lampyridae	Luciolinae	KP763460.1
Sclerotia aquatilis	Lampyridae	Luciolinae	KP763466.1
Pygoluciola dunguna	Lampyridae	Luciolinae	MT106243.1
Pygoluciola qingyu	Lampyridae	Luciolinae	MK292093.1
Curtos bilineatus	Lampyridae	Luciolinae	NC_044789.1
Curtos costipennis	Lampyridae	Luciolinae	AB608764.1
Abscondita terminalis	Lampyridae	Luciolinae	NC_044776.1
Abscondita anceyi	Lampyridae	Luciolinae	NC_039706.1
Emeia pseudosauteri 1	Lampyridae	Luciolinae	MN722654.1
Emeia pseudosauteri 2	Lampyridae	Luciolinae	OK103803
Emeia pulchra	Lampyridae	Luciolinae	OK144132
Luciola italica	Lampyridae	Luciolinae	KM448530.1
Asymmetricata circumdata	Lampyridae	Luciolinae	NC_032062.1
Drilaster axillaris	Lampyridae	Ototretinae	AB608756.1
Drilaster okinawensis	Lampyridae	Ototretinae	AB608758.1
Stenocladius yoshikawai	Lampyridae	Ototretinae	AB608759.1
Lamprigera yunnana	Lampyridae	*incertae_sedis*	MG200082.1
Cyphonocerus marginatus	Lampyridae	Cyphonocerinae	AB608754.1
Rhagophthalmus lufengensis	Rhagophthalmidae	–	DQ888607.1
Rhagophthalmus ohbai	Rhagophthalmidae	–	AB608775.1

strap replicates (Fig. 2). The phylogenetic relationships were displayed using iTOL (v6; https://itol.embl.de/). The new *cox1* sequences from *Emeia* have been deposited in GenBank (accession numbers OK144132 and OK103803).

Results

Phylogenetic analysis

The *cox1* barcode sequences of *E. pseudosauteri* and *E. pulchra* share a 94% sequence identity over the 658 bp segment. The phylogeny constructed from *cox1* of fireflies showed three main clades corresponding to Lampyrinae, Photurinae and Luciolinae (Fig. 2). *Emeia pseudosauteri* was recovered as sister to *E. pulchra* Zhu & Zhen sp. nov. within the subfamily Luciolinae, with strong support (100%).

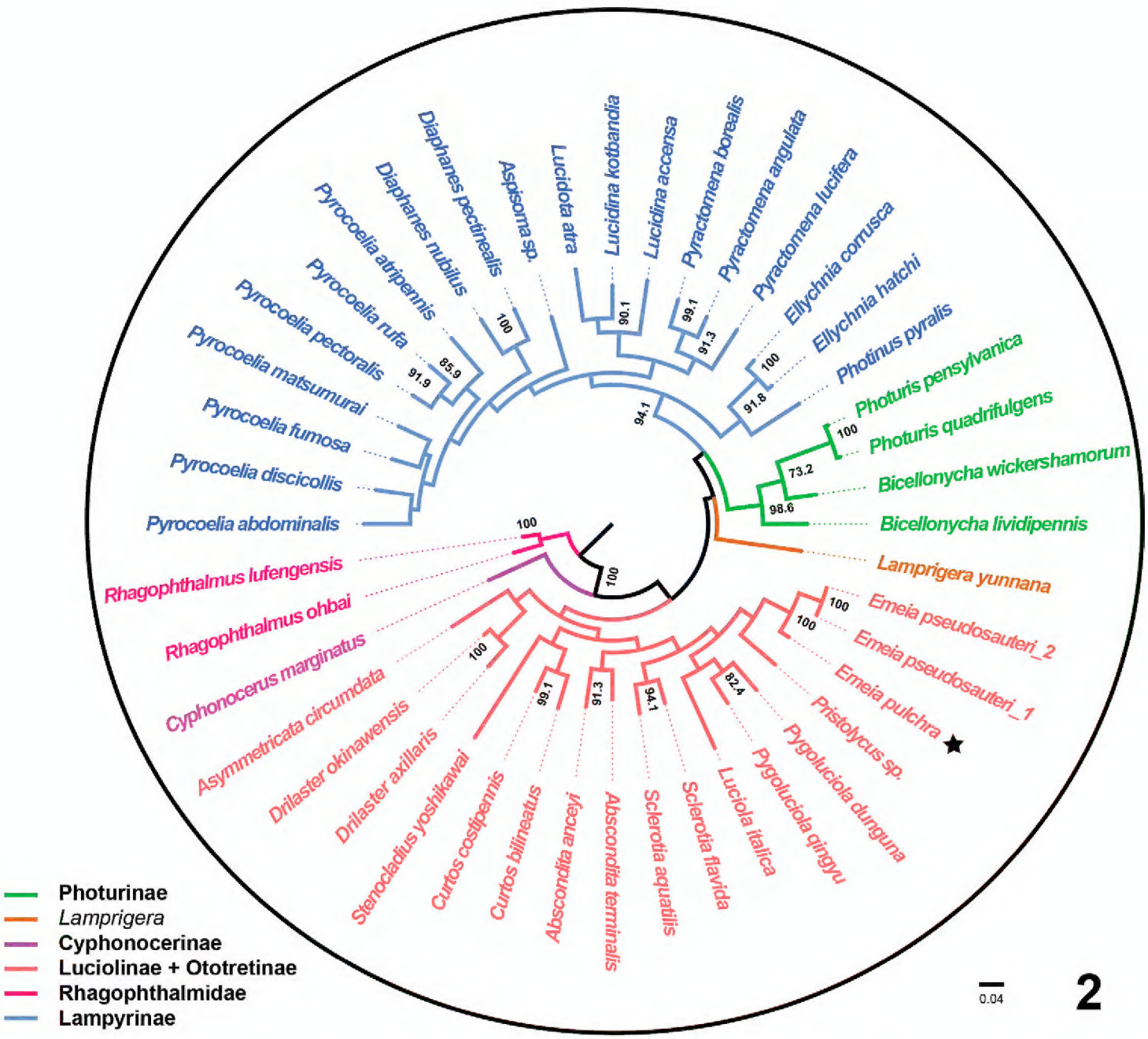


Figure 2. Maximum likelihood *cox1* gene tree of *Emeia* and related genera. The star highlights the new species, *E. pulchra* Zhu & Zhen sp. nov. *Emeia pseudosauteri*_1 was downloaded from GenBank (MN722654.1). *Emeia pseudosauteri*_2 was sequenced during this study. Bootstrap values greater than 0.7 from 1000 replicates are shown.

Taxonomic treatment

Emeia Fu, Ballantyne & Lambkin, 2012

Type species. *Emeia pseudosauteri* Geisthardt, 2004 (designated by Fu, Ballantyne and Lambkin 2012).

Diagnosis (based on adult male). *Emeia* belongs to a group of Luciolinae in which the males have aedeagal parameres widely visible beside the phallus (Ballantyne et al. 2013). *Emeia* differs from *Aquatica wuhana* Fu & Ballantyne, 2010 and *A. lateralis* Motschulsky, 1860, which have black marks on the pronotum (Fu et al. 2010). *Emeia* is distinguished from *Curtos* Motschulsky, 1845, as the species in *Curtos* have a distinctive longitudinal elytral humeral carina and parameres unequal in length (Fu et al. 2012). *Emeia* is closely related to *Pygoluciola* based on our *cox1* phylogeny (Fig. 2), but the two genera can be distinguished by the shape of the pronotum, with median anterior margin gently rounded or slightly medianly emarginate in *Pygoluciola* (Ballantyne and Lambkin 2006) versus lateral margins of pronotum almost parallel in *Emeia*.

Description (based on adult male). Body length 6.5–10.5 mm. Body width 2.7–4.0 mm. Integument black or dark brown, with a narrow (e.g., in *E. pulchra*, see Fig. 3A) or thick (e.g., in *E. pseudosauteri*, see Fig. 8A) black stripe on pronotum.

Head. Hypognathous; head depressed between eyes, eyes exposed in front of pronotum; antennae filiform, with 11 antennomeres (Figs 3B, 8B).

Thorax. Pronotum in dorsal view appearing pink-red or orange-red, with a black median stripe, lateral margins almost parallel (Figs 3A, 8A); surface of elytra smooth, longitudinal carina absent (Figs 3A, 8A); legs long and straight, no femora or tibiae swollen or curved (Figs 3B, 8B).

Abdomen. V2–V5 dark brown or black. Light organs present in V6 and V7, entirely occupying V6; V7 semitransparent (Figs 3B, 8B).

Male genitalia. Trilobate, parameres extending ~0.14 mm (n = 3) beyond phallus; both parameres equal in length (Figs 6A, 12A).

Emeia pulchra Zhu & Zhen, sp. nov.

https://zoobank.org/45330183-64CB-45CE-A2E4-7E013ECECB00

Figs 3–6

Diagnosis (based on adult male). The new species can be differentiated from *E. pseudosauteri* Fu, Ballantyne & Lambkinby the elytron, hindwing venation and aedeagus. In fresh specimens, the elytral apices are black in *E. pulchra* (Fig. 3), but with a narrow orange stripe in *E. pseudosauteri* (Fig. 8). In the male hindwing, the upper vein of the MP_{3+4} venation in *E. pulchra* reaches the margin of the hind wings without

forks (n=2) (Fig. 4). In *E. pseudosauteri*, the upper vein of MP_{3+4} forks and reaches the margin of the hind wings (n=2) (Fig. 10). The aedeagus in *E. pulchra* is approx. 3 times as long as wide (length 1.77 mm: width 0.58 mm) (Fig. 6A), versus approx. 2 times as long as wide (length 1.66 mm: width 0.84 mm) in *E. pseudosauteri* (Fig. 12A).

Description. Male: BL 10.0–10.4 mm; BW 3.5–3.7 mm (three individuals).

Head. Antennae filiform, black, almost 2/3 as long as body length; antennomere 1 cone-shaped; 2 short and cylindrical; 3 to 10 compressed, not bifurcate; 11th antennomere almost 1.5 times longer than 10th, slightly dilated from base to apex. Concave between eyes dorsally in cross section, both eyes occupying about 2/3 width of whole head in ventral view. Eyes spherical, so that head cannot fully contract into pronotum. Mouthparts fully developed, clypeolabral suture flexible, outer edges of labrum reaching inner edges of closed mandibles.

Thorax. Scutellum black and slightly emarginate distally. Elytra elongated, dark brown to black, apices not deflexed in dorsal view, sides slightly convex. Hind wing well developed, r3 half the length of r4 (Fig. 4). Legs long and straight, without swelling on any part, dark brown to black, with dense white hairs.

Abdomen. Dark brown, ventrites gradually diminishing in length posterad. Light organs yellow-white, occupying almost all of V6 and half of V7, not reaching to posterior edges of V7. V6 and V7 rounded laterally (Fig. 5), posterior half of V7 not arched in dorsal view, abruptly narrowed to truncate posterior apex, apex emarginate (Fig. 5C). T7 rounded, without anterolateral corners (Fig. 5A); T8 symmetrical with concealed anterolateral arms, widest across middle with lateral margins subparallel-sided in anterior half, tapering evenly in posterior half to a rounded and partly truncate posterior margin (Fig. 5B). Abdominal spiracles on lateral edges of each abdominal segments. EL/EW = 4.7–4.8; EL/PL= 4.7–5.0 (n=3).

Male genitalia (Fig. 6): Aedeagal sheath (T9, T10, S9) (Fig. 6D, E) 3.15 mm long; anterior half of sternite broad, apically rounded; tergite without protrusion along posterior margin of T9. Aedeagus (Fig. 6A–C) 1.61 mm long. Phallus short (~1.2 mm) and thick, broadest at midlength, becoming thinner at apex and base, parameres (lateral lobes) extending about 0.14 mm beyond phallus. Parameres robust, subparallel-sided, symmetrical, with blunt preapical lateral expansion.

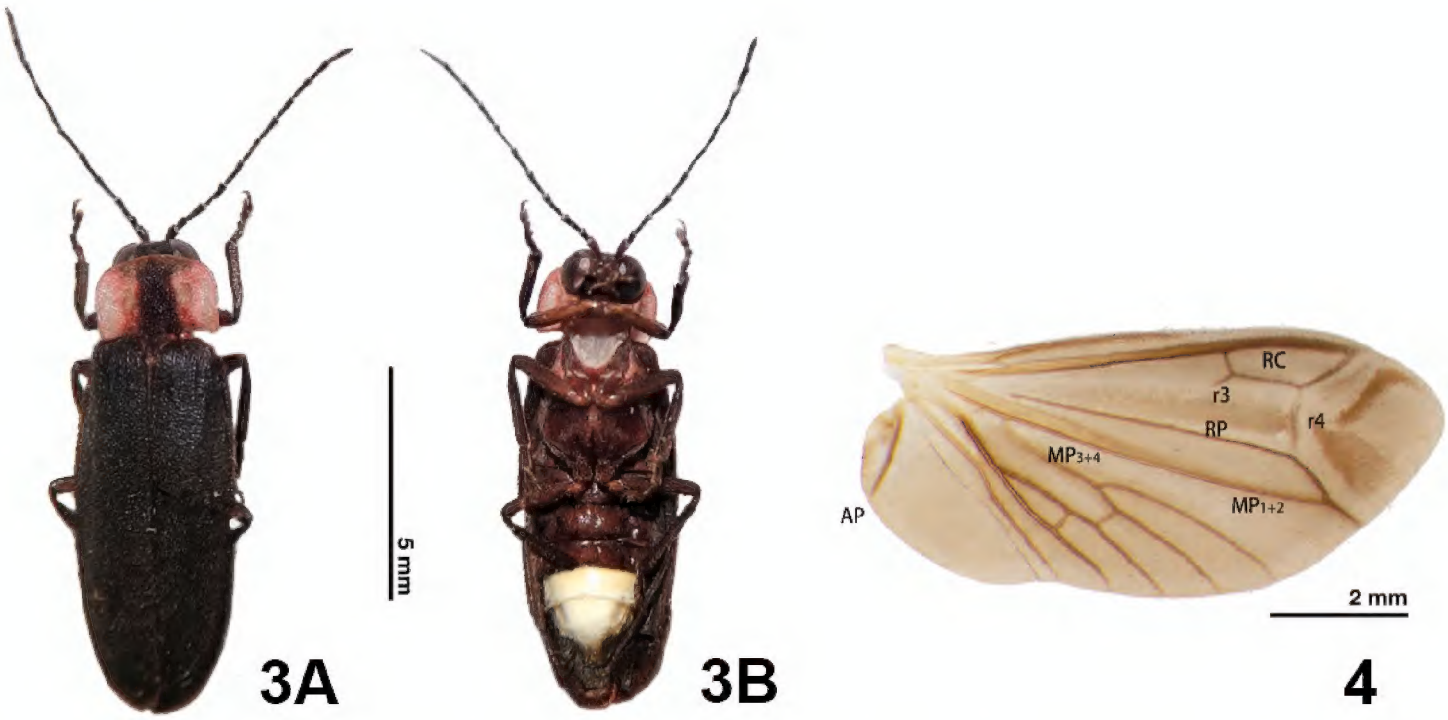
Etymology. The specific name *pulchra* refers to the bright pronotum coloration.

Holotype. China • 1♂; Zhejiang, Lishui; 28°37.56'N, 119°49.7'E; H: 60 m, 2. IV. 2020; Chengqi Zhu leg.; 'HOLOTYPE (red), ♂, *Emeia pulchra* sp. nov., det. Zhu, Zhen, 2021' (Westlake University).

Paratype. China • 1♂; Zhejiang, Lishui; 28°37.56'N, 119°49.7'E; H: 60 m, 2. IV. 2020; Chengqi Zhu leg.; 'PARATYPE (yellow), ♂, *Emeia pulchra* sp. nov., det. Zhu, Zhen, 2021' (Westlake University).

Distribution. China: Zhejiang Province.

Habitat and occurrence. The males were found in an open forest of mainly Chinese wingnut, of the family Juglandaceae [*Pterocarya stenoptera* C. DC.] (Fig.



Figures 3–4. *Emeia pulchra* Zhu & Zhen sp. nov., male **3** habitus of holotype **A** dorsal view **B** ventral view **4** right wing, dorsal view. Scale bars: 5 mm (**3**); 2 mm (**4**).

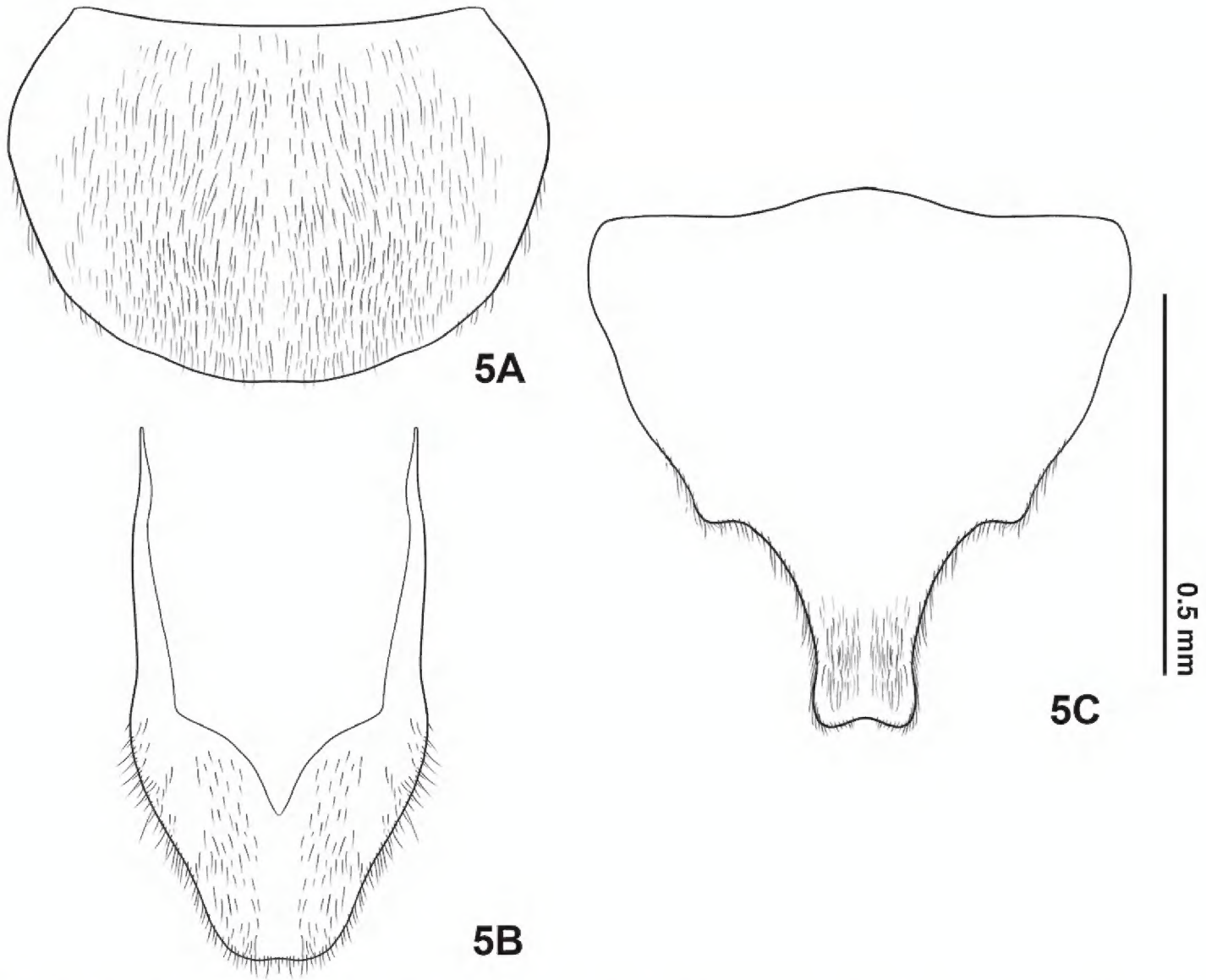


Figure 5. Male abdominal ventrites (V) and tergites (T) of *Emeia pulchra* Zhu & Zhen, sp. nov. **A** T7 **B** T8 **C** V7. Scale bar: 0.5 mm.

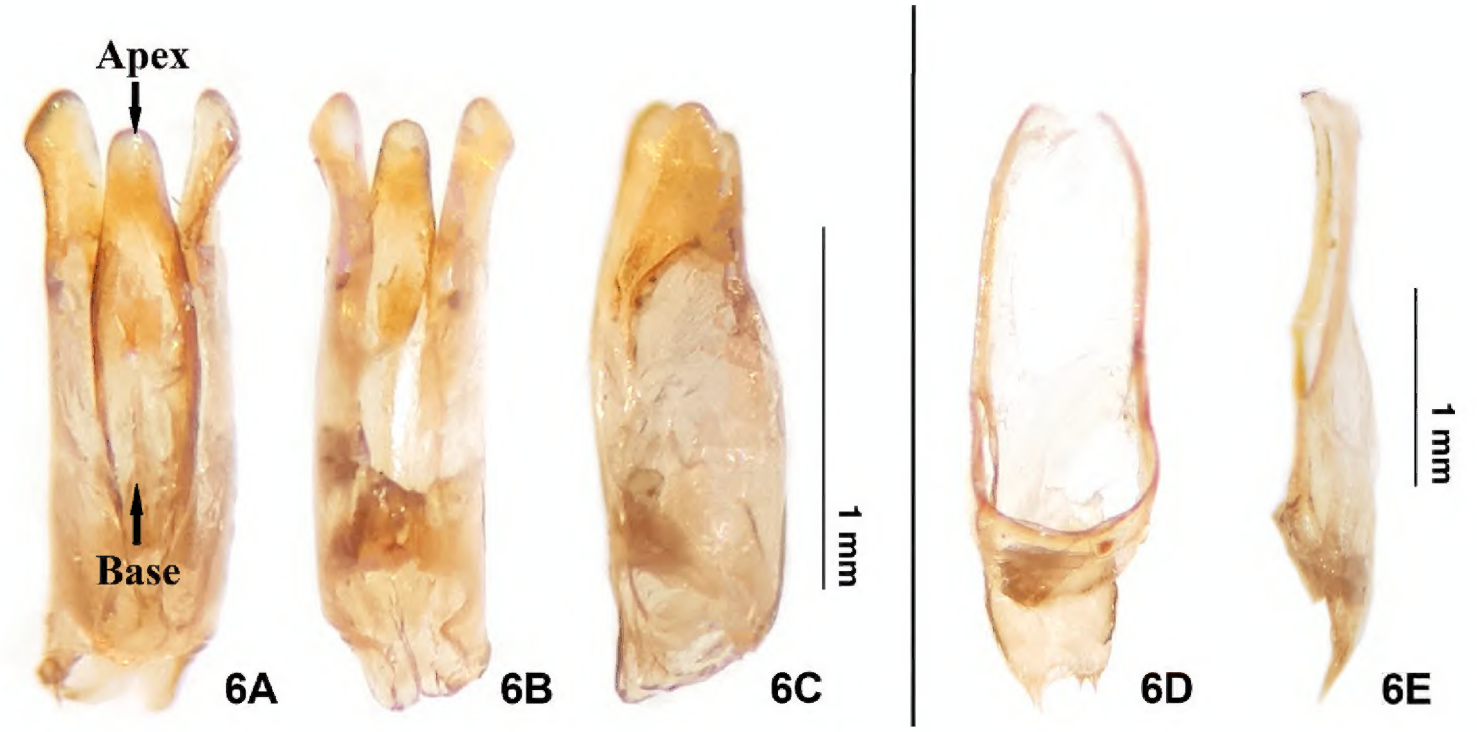


Figure 6. Aedeagusof *Emeia pulchra* Zhu & Zhen sp. nov. **A** dorsal view **B** ventral view **C** lateral view. Aedeagal sheath of *E. pulchra* **D** dorsal view **E** ventral view. Scale bar: 1 mm.

Figure 7. Habitat of *Emeia pulchra* Zhu & Zhen sp. nov. in Jiulong National Wetland Park.

7). The floor of the *Emeia pulchra* habitat was covered with a lush herbaceous layer 20–30 cm high.

There are many terrestrial snails and slugs in this habitat, which may be potential food for *Emeia pulchra* larvae. Combining descriptions from local people and our field observations, adult fireflies are usually observed mid-March. The protection of fireflies has been supported by the Lishui government and Jiulong National Wetland

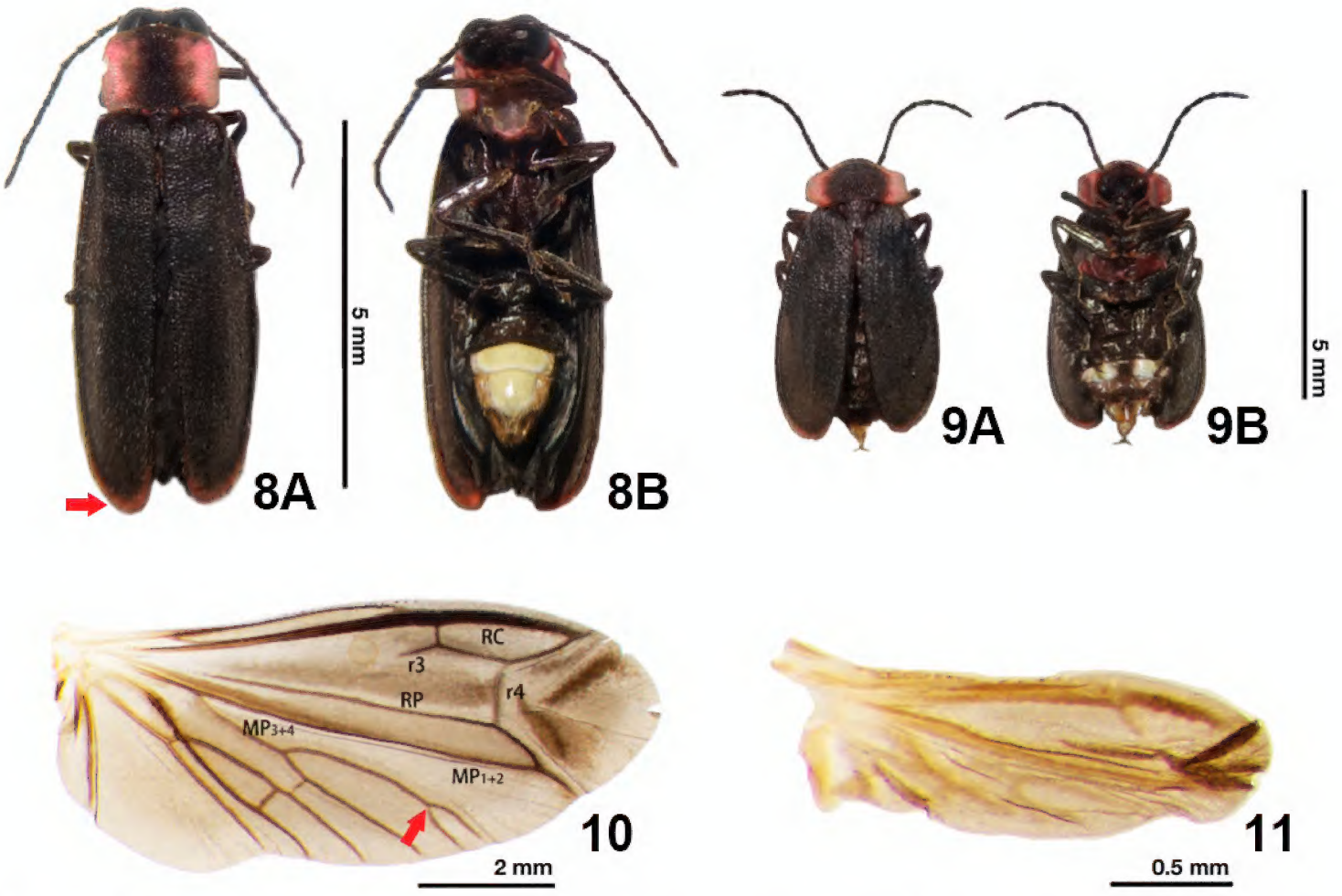
Park management departments, and this area has been protected as Jiulong National Wetland Park (Fig. 7). Fan (2019) reported that the population size of *E. pulchra* has increased from 2014 to 2019 with the protection efforts.

Behavioral remarks. There are two obvious luminous bands at the terminal end of the adult male abdomen. The two bands both emit intermittent bright light during courtship. The male courtship behavior usually starts at 19:00 (approximately 1h after sunset), and peaks at about 20:30. Adult males rest on higher herbs and emit yellow and green flashing light. Males are reluctant flyers; the distance of each flight ranges from 0.5 to 5 m.

Emeia pseudosauteri (Geisthardt 2004)

Figs 8–12

Emeia pseudosauteri (Geisthardt 2004). *Zootaxa* (3403), 1–53. TL: 'Mt. Tian Tai, Sichuan Province, China'.



Figures 8–11. *Emeia pseudosauteri* Fu, Ballantyne & Lambkin, 2012. Male and female **8** habitus of male **A** dorsal view. Arrow highlights narrow orange stripe on elytral apices. The color appears darker in this photo, but it is orange and easily seen in both dried and fresh samples **B** ventral view **9** habitus of female. **A** dorsal view **B** ventral view **10** right wing of male. Dorsal view. Arrow points to wing venation, which differs between the two *Emeia* species **11** right wing of female. Dorsal view. Scale bars: 5 mm (**8, 9**); 2 mm (**10**); 0.5 mm (**11**).

Specimens examined. China: 6♂♂,1♀, Sichuan, Mt. Tian Tai, 3.IV. 2021, Chengquan Cao leg. We herein examined specimens of *E. pseudosauteri* from Mt. Tian Tai (the type locality), and their identity was further verified using *cox1* barcode sequences (Fig. 2) and morphological examination (Figs 8–12).

Figure 12. Aedeagus of *Emeia pseudosauteri.* **A** dorsal view **B** ventral view **C** lateral view. Male aedeagal sheath of *E. pseudosauteri* **D** dorsal view **E** ventral view. Scale bars: 1 mm.

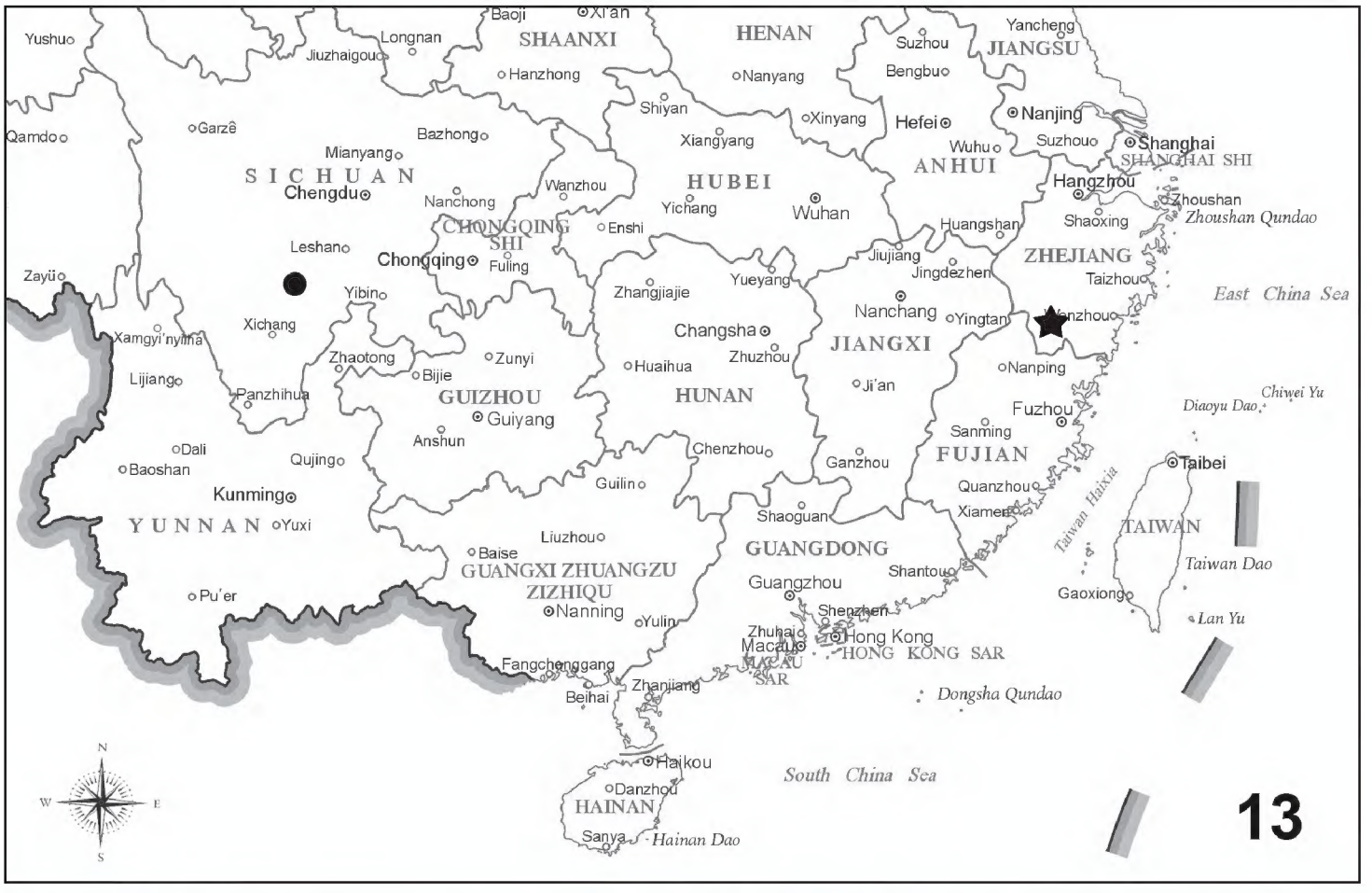


Figure 13. Distribution map of the genus *Emeia* in China. The black star indicates *E. pulchra* Zhu & Chen sp. nov., the black dot *E. pseudosauteri* (map of China from: http://bzdt.ch.mnr.gov.cn/).

Key to species (adult males)

1 The elytral apices have a narrow orange stripe in both fresh and dried specimens; upper vein of MP_{3+4} forked and reaching edge of hind wing (Fig. 10); phallus and parameres broad, 2 times as long as wide (Fig.12A)..... ***E. pseudosauteri* Fu, Ballantyne & Lambkin**

– The elytral apices are black in fresh and preserved specimens (Fig. 3A); upper vein of MP_{3+4} reaching margin of hind wings, but without forks (Fig. 4); phallus and parameres slender, 3 times as long as wide (Fig. 6A)..... ***E. pulchra* Zhu & Zhen, sp. nov.**

Discussion

In this study, we summarized the diagnostic features of the genus *Emeia*. *Emeia pulchra* Zhu & Zhen, sp. nov. is morphologically similar to *E. pseudosauteri* Fu, Ballantyne & Lambkin, 2012 from Sichuan Province. However, we found differences in the antennal length and body size between the two species. The body size of a species may vary due to nutrition and environmental factors, so we did not include size in the diagnosis to the new species. The antenna of male *E. pulchra* (Fig. 3) is narrower than that of *E. pseudosauteri* (Fig. 8) in lateral view. Females of *E. pseudosauteri* have body length about 2/3 of that of the male and have normal elytra (Fig. 9), but their hind wings are small and shrunken, about 1/4 length of the male hind wings (Figs 10, 11). In the male, we found that the hind wing of *E. pseudosauteri* was relatively narrower and longer than that of *E. pulchra*. The elytral apice has a narrow orange stripe in both fresh and dried specimens of *E. pseudosauteri*, whereas it is black in *E. pulchra* (in three *E. pulchra* and six *E. pseudosauteri* examined). The observed body size of *E. pseudosauteri* (BL 6.6–7.2 mm; BW 2.7–2.9 mm; six individuals measured) was smaller than for *E. pulchra* (BL 10.0–10.4 mm; BW 3.5–3.7 mm; three individuals measured). In the male genitalia, the aedeagus of *E. pulchra* (Fig. 6A) is narrower than that of *E. pseudosauteri* (Fig. 12A), and the parameres are less curved (Figs 6B, 12B). In addition, the new species is only known from S. Zhejiang, whereas *E. pseudosauteri* is only found 1600 km westward, in the Sichuan Province (Fig. 13).

The "barcode region" of *cox1* is often used as an aid to new species' identification and distinction from close relatives in the Barcode of Life Data system (Ratnasingham and Hebert 2007; Lin et al. 2009). Currently, this method has been widely and successfully used to identify closely-related species and conspecific individuals. Our *cox1* gene tree recovered the major subdivisions within Lampyridae, including Lampyrinae, Photurinae and Luciolinae. This tree is consistent with recent studies using 436 loci (Martin et al. 2019) or 15 mitochondrial genes (Chen et al. 2019), and supports that the placement of *Emeia* in Luciolinae (Fig. 2). Both the *cox1* tree and morphology support *E. pulchra* as the closest sister species of *E. pseudosauteri*.

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